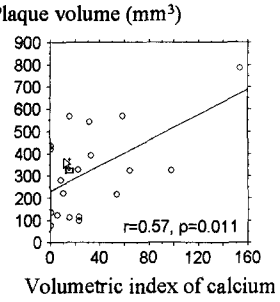
than the non-stenotic segments, there is volumetrically more calcium contained in the non-stenotic arterial segments. Plaque volume correlated strongly with a volumetric index of arterial calcification .



ORAL CONTRIBUTIONS

816 New Applications of Microbubbles

Monday, March 18, 2002, 11:00 a.m.-12:15 p.m.
Georgia World Congress Center, Room 255W

11:00 a.m.

816-1 Development of an Angiogenesis-Targeted Microbubble Ultrasound Contrast Agent

Howard Leong-Poi, Alexander L. Klibanov, Jonathan P. Christiansen, Yuqing Huo, Jonathan R. Lindner, *University of Virginia, Charlottesville, Virginia.*

Background. An accurate non-invasive technique for assessing angiogenesis in patients is not available. We hypothesized that microbubbles targeted to α_v-integrins, which are expressed by endothelial cells in response to growth factors, would be retained in angiogenic vessels.
Methods. Microbubbles targeted to α_v-integrins were prepared by conjugating either monoclonal antibodies against murine α_v-integrins (MB_α), or the disintegrin echistatin (MB_E), to the shell surface. Control microbubbles bearing an isotype control antibody (MB_{iso}) were also prepared. Flow cytometry was performed to assess microbubble attachment to cultured murine endothelial cells (MEC) that express α_v-integrins when activated by IL-1β. The microvascular behavior of these microbubbles was assessed by intravital microscopy of 5 mice following intrascrotal placement of heparin-alginate pellets for time release of 1.5 μg of basic fibroblast growth factor (bFGF) over 5 days, and in 3 sham-treated control mice. Observations were made following venous injections of 1×10^7 targeted or control microbubbles. Vascular density was assessed following intravenous injection of FITC-dextran.
Results. Flow cytometry demonstrated that MB_α and MB_E, but not MB_{iso}, adhered to activated MEC that express α_v-integrins. On intravital microscopy, bFGF-treated mice demonstrated increased cremasteric vascular density compared to controls. Microbubble retention in control mice was uncommon (4 ± 2 mm^{-3}) for all microbubbles, and was mostly due to venular leukocyte attachment. In bFGF treated animals, microbubble retention was greater ($p<0.05$) for MB_α (13 ± 9 mm^{-3}), and MB_E (13 ± 8 mm^{-3}), compared to MB_{iso} (1 ± 1 mm^{-3}). Enhanced retention of MB_α and MB_E was due to direct endothelial attachment predominantly in arterioles, with some attachment in capillaries and venules.
Conclusions. Microbubbles targeted to endothelial α_v-integrins are retained in vessels stimulated by bFGF. Microbubbles targeted to endothelial α_v-integrins may provide a means to non-invasively assess angiogenesis using contrast-enhanced ultrasound imaging.

11:15 a.m.

816-2 Myocardial Contrast Echocardiography Can Be Used to Detect Acute Heart Transplant Rejection

Erxiong Lu, Melissa M. Black, David Fischer, William R. Wagner, Flordeliza S. Villanueva, *University of Pittsburgh, Pittsburgh, Pennsylvania.*

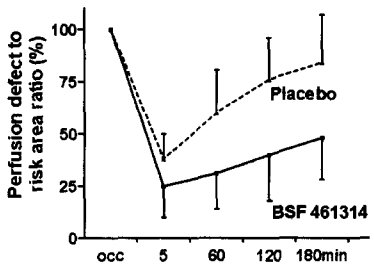
Background: Albumin microbubble (mbub) adhesion to activated leukocytes provides an approach to ultrasonic identification of inflamed endothelium. Because acute heart transplant rejection (REJ) generates a strong myocardial inflammatory response, we hypothesized that myocardial contrast echocardiography (MCE) can detect REJ.
Methods: Heterotopic heart transplant was performed to the abdominal aorta of Lewis rats. Rat donor strains were Brown Norway (REJ group, n=18) or Lewis (Control group, n=11). On post-op day 5, rats were given i.v. 0.05ml albumin mbub. Mbub retention was detected by intermittent transthoracic high mechanical index harmonic MCE imaging of the transplant to capture 4 frames spaced 300msec apart at both 2.5 and 3 minutes after injection. Rats were killed after 3 injections and post-mortem H&E staining was performed for histologic REJ grade. MCE background-subtracted videointensity (VI) was measured in the entire left ventricular (LV) myocardium, LV areas with greatest histologic lymphoid infiltration, and the right ventricle. Data are expressed as VI difference between the first 2.5-minute frame (adhered+circulating mbub) and 3-minute frame (circulating mbub), equivalent to the VI attributable to adhered mbub.
Results: Three rats died; 8 were excluded due to segmental infarction, attenuated images, or inflammation in a Control. All remaining rats in the REJ group (n=12) showed histologic REJ, compared to none in Controls (n=6). LV VI was higher in the REJ vs Controls, both for the entire myocardium (11±3 vs 5±1, $p<0.04$), as well as those regions paralleling severe inflammation (12±2 vs 4±1). There was a trend towards higher right ventricular VI in the REJ rats, and higher VI in rats with severe compared to mild REJ.
Conclusions: In this heart transplant model, persistent myocardial enhancement during MCE is a marker of acute REJ. These data are consistent with mbub retention in the microcirculation of REJ heart, and are the first demonstration of MCE imaging of myocardial inflammation. The use of mbub specifically targeted to inflammatory endothelial markers might augment such imaging. MCE may thus permit diagnosis of heart disease associated with endothelial activation.

11:30 a.m.

816-3 Dynamics of Ischemia-Reperfusion Injury Evaluated by Real-Time Myocardial Contrast Echocardiography: Protective Effects of a Novel Endothelin A Antagonist BSF 461314

Alexander E. Hansen, Raffi Bekeredjian, Arthur Filusch, Marie-Luise Gross, Klaus Muenter, Marie Gebhardt, Anne Broillet, Helmut Kuecherer, *Cardiology, Heidelberg, Germany.*

Recanalization of the infarct-related artery does not guarantee myocardial salvage due to reperfusion injury of microvessels. Real-time myocardial contrast echocardiography (MCE) allows visualisation of spatial and temporal dynamics of infarct expansion.
Methods: 12 open-chest pigs underwent 45 min of mechanical LAD occlusion followed by 180 min of reperfusion. A new highly selective endothelin A antagonist BSF 461314 (20 mg) or placebo was given intravenously 20 min before releasing LAD occlusion. MCE was performed using Power Pulse Inversion imaging (PPI) at MI=0.09 during steady state intravenous infusion of Sonovue (90ml/h). Perfusion-defect-to-risk-area-ratio and contrast replenishment kinetics (A=peak intensity as % of baseline intensity, β=rate of intensity rise) were measured at baseline, during occlusion (occ) and following reperfusion and compared to histology.
Results: During LAD occlusion signal intensities were reduced in anterior regions (A=4.2±3%, β=0.05±0.02) defining risk areas and approached baseline levels 5 min post recanalization (A=102±40%, β=0.58±0.16) but gradually decreased during reperfusion (A=37±12%, β=0.18±0.13; $p<0.005$). Accordingly, the defect-to-risk-area-ratio progressively increased during reperfusion. This increase however was markedly reduced in the BSF treated group (see fig., $p=0.004$).
Conclusions: Real-time MCE can evaluate dynamics of myocardial reperfusion injury and monitor cardioprotective effects of endothelin A antagonism.



11:45 a.m.

816-4 Albumin Microbubbles Can Be Targeted to Activated Neutrophils In Vivo: Application to Assessment of the Neutrophils Infiltration Pattern Produced by Ischemia/Reperfusion

Isao Kondo, Koji Ohmori, Akira Oshita, Hiroto Takeuchi, Kaori Shinomiya, Yang Yu, Yuichiro Takagi, Kazushi Yukiiri, Yoshihiro Wada, Katsufumi Mizushige, Masakazu Kohno, *kagawa Medical University, Kagawa, Japan.*

Background: Contrast enhancement at the site of inflammation has been attributed to the interactions between activated neutrophils (NP) and microbubbles (MB) including adhesion and phagocytosis. Intensity of signal from MB with lipid shell was correlated with the degree of inflammation in reperfused whole kidneys in mice. However, a direct comparison of spatial pattern of NP infiltration with that of contrast enhancement has not been reported. The ability of MB with albumin shell to detect and locate the activated NP in the site of inflammation remains unknown. Therefore, in an ischemia/reperfusion model, we correlated the regional signal intensities from albumin shell MB with the distribution of activated NP in the tissue, assessed with immunohistochemistry. Methods: In seven SD rats unilateral total renal ischemia (20 min) and reperfusion (4 hours) were implemented. Long axis harmonic (1.8/3.6MHz) images of the reperfused and control kidneys were obtained by an S4 transducer of SONOS5500 with MI of 1.1 before, during, and 10 min after the intravenous infusion of 10% Optison (1mL/5min). Ultrasound emission was limited only to obtain two frames each time. Then, immunohistochemical staining of NP elastase was performed to assess the distribution of the activated NP on the tissue slices corresponding to the ultrasound images. Results: On the first frame of the image during MB infusion, dense opacification was observed both in reperfused and control kidneys in all animals. On the first frame at 10 min after the infusion, CI was significantly reduced both in medulla (2±1) and cortex (2±1) in control kidneys. In contrast, significant signal enhancement was observed in reperfused kidneys, in which CI was significantly higher in medulla (23±13) than in cortex (10±8, $p<0.05$ vs. medulla). Quantitative histological